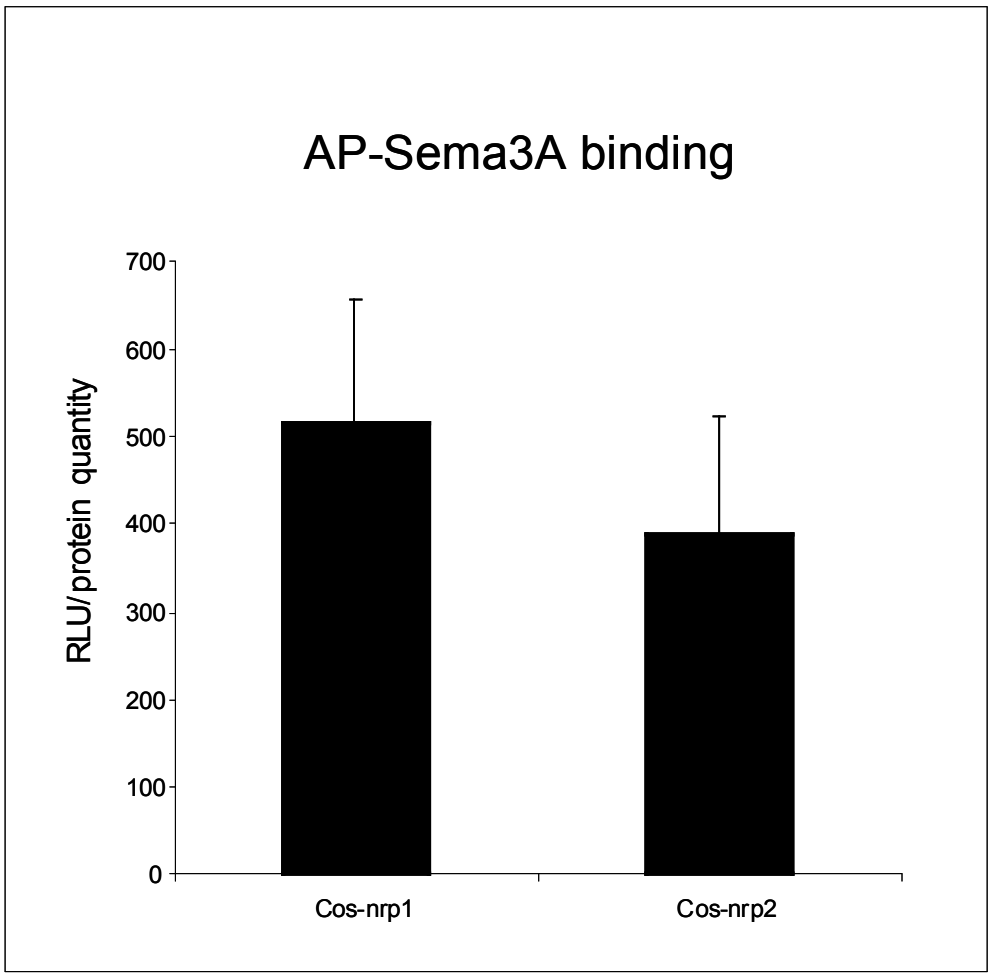
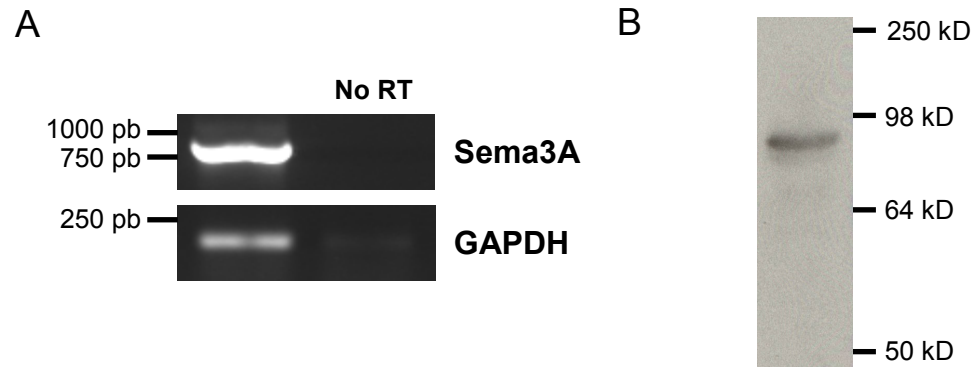
Supplementary figure 1:

Binding assay:

Binding assay was performed as previously described. In brief, wild-type COS-1 cells, NRP1-expressing COS-1 cells (COS-NRP1) or NRP2 expressing COS-1 cells (COS-NRP2) were cultured on 96-well plates (Perkin Elmer Life Science, Massachusetts, USA) previously coated with poly-L-lysine (0.005 mg/ml). Cells were washed with serum-free medium and incubated with conditioned medium containing alkaline phosphatase-coupled Sema3A (AP-Sema3A) obtained from AP-Sema3A stably expressing HEK cells for 2h at 4°C (Bagnard et al., 1998). Cells were washed with PBS and fixed in 4% formaldehyde for 15 min. After three washes in PBS, the plate was warmed for 50 min at 65°C. Cells were subsequently incubated with 80 µl of alkaline phosphatase luminescent substrate (Lumi-phos, Lumigen, Inc) to determine luminescence after 15 min with Microlumat Plus system (Berthold Technologies). Statistical analysis was performed by using Student's t test.



Supplementary figure 2



C6 glioma cells express Sema3A

A) RT-PCR

Total RNA was extracted using RNEasy mini kit (Qiagen) and reverse transcribed 1 h at 42°C using superscript first strand synthesis system (Invitrogen). PCR amplification consisted in 3 min denaturation at 94°C followed by 30 cycles of 30 sec denaturation at 94°C, 30 sec annealing at 58°C and 1 min extension at 72°C. The amplification products were resolved on 1% agarose gel. The following primers were used: GAGGGAGCAGGATTAGAGTC as the Sema3A forward primer and AGAGAGGCAGTCAGTAGTTTGGG as the Sema3A reverse primer.

B) Western Blot analysis

The culture medium was removed, C6 cells washed with PBS, collected with a cell scraper and centrifuged at room temperature for 5 min at 100G. The pellet was suspended in lysis buffer [150 mM NaCl, 0.1 % sodium dodecyl sulphate, 50 mM Tris-HCl, pH 8, 1mM EDTA, 1 % Nonidet P-40, 0.5 % sodium deoxycholate] and frozen for 5 min at – 20°C before sonication for 30 s. The protein content was determined using the Bradford method; the samples were subjected to electrophoresis on sodium dodecyl sulphate gel (10 %) and electro-transferred to a nitrocellulose membrane. After transfer, the membrane was washed for 5 min at room temperature in TBS-Tween 0.1 % and blocked for 1h in Tris-buffer containing 0.1 % Tween 20 and 3 % milk. The blots were incubated overnight at 4 °C with gentle agitation with the primary antibody anti-Semaphorin 3A (1:2000; rabbit polyclonal antibody raised against amino acids 103-402 of Semaphorin 3A of human origin; Santa Cruz Biotechnology, Santa Cruz, CA)[24], rinsed and washed to be incubated for 1 h at room temperature with HRP-labeled secondary antibody (1:2000; P.A.R.I.S.). Bound antibodies were detected using an ECL western blotting detection kit (34076, Pierce).